

## Original Article

# Salivary immunoglobulin classes in Nigerian cigarette smokers: Indication for increased risk of oral diseases

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## ABSTRACT

**Background:** Cigarette smoking is a worldwide social epidemic and it is one of the main causes of preventable death and disability. Gingivitis, periodontitis, pocket depth, attachment loss, alveolar bone loss, and tooth loss are some of oral pathologies commonly found in cigarette smokers. The aim of this study was to explore, for the first time among Nigerians, the interplay between components of cigarette smoke and salivary levels of immunoglobulin classes so as to provide oral immunological based reasons for oral diseases in cigarette smokers.

**Materials and Methods:** In this case-control study, 5 mL of unstimulated saliva was collected in plain sample bottles from 24 active smokers who smoke at least 6 sticks of cigarette per day and 21 sex and age-matched non-smokers who were apparently healthy. The samples were spun and supernatant stored at -20°C until assayed. The immunoglobulin levels of the samples were estimated using enzyme-linked immunosorbent assay (ELISA). Student's *t*-test (unpaired) was used to determine significant differences between the two groups. *P* values less than 0.05 was considered significant.

**Results:** No significant differences were observed in the mean salivary levels of IgG, IgA, and IgE. Only IgM was significantly lower in smokers compared with non-smokers ( $P = 0.038$ ). The proportion of smokers with detectable level of salivary IgE was lower compared with controls.

**Conclusion:** Our study showed that there is decreased salivary IgM in smokers. This observation suggests that reduced salivary immunoglobulin level of IgM might be involved in the pathogenesis of oral diseases in cigarette smokers.

**Key Words:** Cigarette, immunoglobulin, nicotine, pan-hypogammaglobulin, saliva

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## INTRODUCTION

Tobacco smoke is a complex mixture of more than 4500 chemicals, many of which have toxic and/or carcinogenic activity.<sup>[1]</sup> Some of the components, which could be in the form of gases, vapors, and particulates, include carbon monoxide, hydrogen cyanide, phenols, acrolein, ammonia, formaldehyde, nicotine, nitrosamine, tar, heavy metals, and at least 48 known cancer-producing substances.<sup>[2]</sup>

Cigarette smoking is a worldwide social epidemic and it is one of the main causes of preventable death and disability.<sup>[1]</sup> It is an established risk factor for premature mortality due to cancer, cardiovascular disease, and chronic obstructive pulmonary disease.<sup>[3]</sup> The increased susceptibility of cigarette smokers to infections reflects multifunctional alteration of their innate and adaptive immune responses.<sup>[1,3]</sup>

Acrolein, a toxic unsaturated aldehyde, affects neutrophil functions and thus increases susceptibility to lung infections.<sup>[4]</sup> Leukocytosis is a well-known effect of cigarette smoking though the function of these cells is greatly reduced.<sup>[5,6]</sup> Eichel *et al.*<sup>[7]</sup> reported that a single cigarette provided enough toxic material that completely inhibited the function of oral salivary neutrophils *in situ*. Reduced phagocytic activity of neutrophils was also reported in smokers,

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which could be responsible for decreased defence of the gingival against bacterial attack.<sup>[8,9]</sup>

Continued exposure to cigarette smoking has been shown to affect both humoral and cellular immune responses. Initially (hours to days), there is an acute depression of the immune response followed by stimulation (weeks to months), and finally depression of the immune system sets in. This causes a decreased response to antigens and reduced serum concentration of IgG, IgM and IgA,<sup>[10]</sup> and also increased levels of autoantibodies notably; anti-nuclear rheumatoid factors.<sup>[11,12]</sup>

Although several reports have shown that cigarette smoking significantly reduces serum levels of immunoglobulin classes in humans,<sup>[10,13]</sup> a recent report by Arinola *et al.*<sup>[14]</sup> showed significantly elevated serum levels IgG and IgM in active smokers. Similarly, conflicting observations have been reported on levels of salivary immunoglobulin classes in smokers. Bennet and Read<sup>[15]</sup> reported significantly reduced level of salivary IgA in smokers while EngstrÅm and EngstrÅm<sup>[16]</sup> reported increased levels of salivary IgA in smokers.

Studies on serum levels of immunoglobulin classes in smokers are conflicting. More so, research on salivary levels of immunoglobulin classes in cigarette smokers has been rarely explored. To explore, for the first time among Nigerians, the interplay between components of cigarette smoke and salivary levels of immunoglobulin classes, our study estimated salivary immunoglobulin classes (IgG, IgA, IgM, IgE) in Nigerian smokers to provide oral immunological based reasons for oral diseases in cigarette smokers.

## MATERIALS AND METHODS

Forty-five (45) subjects were recruited for this study after obtaining informed consent from each subject and an ethical approval from the University of Ibadan/University College Hospital (U.I/U.C.H) Joint Ethics Review Committee. The test group consisted of 24 active smokers who smoke at least 6 sticks of cigarette per day while the control group comprised 21 sex- and age-matched non-smokers who were apparently healthy. All subjects were screened of periodontitis and other oral diseases. Those with oral diseases were excluded from the study. Also excluded were individuals with pregnancy, diabetes, and human immunodeficiency virus (HIV) infection.

A short structured questionnaire was administered on each subject to obtain information on age, sex, occupation, cigarette smoking, and drug consumption.

About 5 mL of unstimulated saliva was collected from each subject using the spitting method into plain sample bottles. The samples were collected between 9 am and 11 am, at least 1 h after eating or washing of mouth. The samples were centrifuged at 3000× *g* for 5 min and the clear supernatant gently pipetted out into another clean plain bottle and stored at -20°C until analysed.

Immunoglobulin levels were estimated using enzyme-linked immunosorbent assay (ELISA) supplied by Immunology Consultant Laboratory, USA. IgE kit was supplied by Leinco Technologies, USA. The assay was carried out following manufacturer's instructions.

## Statistical analysis

The data were presented as mean and standard deviation. Student's *t*-test (unpaired) was used to determine significant difference between the means. The 5% ( $P < 0.05$ ) level of significance was considered significant.

## RESULTS

The mean ages of smokers and non-smokers were 39.9 and 39.5 years, respectively. No significant differences were observed in the mean salivary levels of IgG, IgA and IgE. Only IgM was significantly low in smokers compared with non-smokers ( $P = 0.038$ ).

The mean level of salivary IgE was lower in smokers compared with control. Only 1 smoker (4.17%) had a detectable level of salivary IgE (0.04) while two non-smokers (9.52%) had detectable levels of IgE (0.24). Also the proportion of smokers with detectable level of salivary IgE was lower compared with controls [Table 1].

**Table 1: Levels of salivary immunoglobulin classes in active smokers and non-smokers**

Immunoglobulins	Smokers (n=24)	Non-smokers (n=21)	P value
IgG (ng/L)	355.7±40.8	369.6±44.6	0.454
IgA (ng/L)	570.4±145.6	596.9±103.6	0.497
IgM (ng/L)	703.1±169.3	779±49.5	0.038*
IgE (IU/mL)	0.04±0.2	0.24±0.89	0.417

\*Significant at  $P < 0.05$

## DISCUSSION

Cigarette smoking is among social practices commonly found in some Nigerian youth, despite its adverse health consequences.<sup>[6]</sup> Gingivitis, periodontitis, pocket depth, attachment loss, alveolar bone loss, and tooth loss are some of oral pathologies commonly found in cigarette smokers.<sup>[9]</sup> Tobacco smoking predisposes to infection, emphysema, and lung cancer. Herr *et al.*<sup>[17]</sup> reported that current or former smoking is associated with reduced levels of human  $\beta$ -defensins 2 (hBD2) in pharyngeal washes and sputum of patients with acute pneumonia. Of note, smoking is associated with reduced levels of surfactant proteins A and D (SP-A and SP-D).<sup>[18,19]</sup>

IgA is the predominant immunoglobulin secreted into external secretions including saliva and tears. Its increase could be due to increased local infection, increased antigenic inflammatory stimulus, increased local synthesis, and local host reaction against the presence of disease.<sup>[20]</sup> Levels of other immunoglobulin classes (IgG, IgM and IgE) have also been reported to decrease in oral diseases as observed in gingival fluid exudates.<sup>[10]</sup>

The mean levels of all the immunoglobulin classes were reduced in the saliva of smokers compared with non-smokers. However, only the salivary IgM mean level was significantly reduced in smokers ( $P = 0.038$ ). This observation contradicts the report of Engström and Engström<sup>[16]</sup> who observed increased salivary IgA only. They suggested that their observation could be a reflection of protection of the oral mucosa. Earlier report by Bennet and Read<sup>[15]</sup> who reported a significantly low salivary level of IgA only partially supports our observed low salivary IgA level in smokers. In our study, the exclusion of subjects with oral diseases could be responsible for the observed differences in salivary IgA as there could be upsurge in oral antibodies production consequent to oral infection or diseases. More so, our observation could be as a result of nicotine contained in cigarette as nicotine affects the exocrine glands by an initial increase in salivary secretions followed by inhibition of the secretions.<sup>[9]</sup>

A study carried out on patients with oral mucosal disease showed higher level of salivary IgG. The causative effect was suggested to be increased permeability of oral mucosa which made it easy for the passage of IgG from vascular and extra vascular compartment into saliva by passive transmucosal diffusion.<sup>[20,21]</sup> Ferson<sup>[13]</sup>

had earlier reported reduced serum immunoglobulin classes in smokers. Experimental studies also showed that mice that were chronically exposed to cigarette smoke were more susceptible to influenza and murine sarcoma viruses.<sup>[22,23]</sup> Similarly, enhanced replication of influenza virus and *Legionella pneumophila* was observed in the lungs of nicotine-treated animals and cells lines, respectively.<sup>[24,25]</sup>

Based on this observation, therefore, it could be suggested that reduced salivary immunoglobulin levels, especially IgM, could play an important role in the pathogenesis of oral diseases in cigarette smokers and thus could have potential benefit in screening smokers at risk of developing oral diseases.

Although only salivary IgM was significantly low in smokers, the observed non-significant reduction in all the classes of salivary immunoglobulin suggests pan-hypogammaglobulin in them. This supports the widely accepted view that cigarette smoke suppresses the immune system.

Further study is required to provide explanation for the reported blood polyclonal B cells activation<sup>[14]</sup> and the significantly reduced salivary IgM levels observed in these cigarette smokers.

Small sample size (due to strict inclusion criteria), unsuitable samples (occasioned by stimulation or collected after 11am), and exclusion of Ziehl-Neelson positive patients were some of the limitations of this study.

## CONCLUSION

Our study showed that there is decreased salivary IgM in smokers. This observation suggests that reduced salivary immunoglobulin level of IgM might be involved in the pathogenesis of oral diseases in cigarette smokers.

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